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Relative toxicity of phorate and its metabolites to Tetranychus urticae Koch (Tetranychidae; Acarina) and Empoasca fabae Harris (Cicadellidae; Homoptera)

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RELATIVE TOXICITY OF PHORATE AND ITS METABOLITES TO TETRANYCHUS URTICAE KOCH (TETRANYCHIDAE; ACARINA) AND EMPOASCA FABAE HARRIS (CICADELLIDAE; HOMOPTERA)

by

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INTRODUCTION

Phorate (0,0 diethyl-S-thiomethyl phosphorodithioate) is one of the organophosphates currently used as a phytosystemic insecticide. Because its phytosystemic properties enhance its selective use against phytophagous pests and its persistence is brief (relative to most chlorinated hydrocarbon insecticides), it is being considered along with other organophosphates and carbamates as an alternative to the chlorinated hydrocarbons in pest control. Use of chlorinated hydrocarbons has brought about increasing concern over persistent residues which effect slow but cumulative contamination of the environment and also exert a continuous selective pressure upon pest populations, resulting in the development of pesticide-resistant insect strains.

When applied to seeds, roots, foliage, or implanted into trunks of trees, phorate is translocated to various parts of the plants and undergoes metabolic changes to a series of cholinesterase-inhibitory compounds toxic to chewing and sucking insects.

When a susceptible pest ingests plant juices containing the systemic insecticide, a complex with cholinesterase enzyme is formed. This phosphate-enzyme complex is only slowly hydrolized, thus blocking the enzyme action for acetylcholine decomposition to form acetic acid and choline. Acetylcholine, a chemical transmitter, is released at nerve endings when an impulse is propagated along an axon and reaches a synapse. The impulse propagated bridges the synapse via acetylcholine and triggers an appropriate response through muscle, gland, or another axon. After the impulse has passed the acetylcholine bridge, the enzyme cholinesterase promptly eliminates acetylcholine at the cholinergic junction and synapse sensitivity is restored.

However, inhibition of the enzyme (e.g. reaction with organophosphorous insecticide) results in acetylcholine accumulation at nerve endings that leads into excessive activity and subsequent blockade at the synapse. Symptoms of organophosphate-poisoning in insects include excitability, tremors (especially noticeable in the extremities), and finally paralysis and death.

Chemical change of phorate in the plant and/or in the soil is predominantly oxidative in nature. Oxidation of the thioether sulfur of phorate to the sulfoxide and sulfone is an important activation process. The oxidation products are more potent cholinesterase inhibitors than is phorate. However, further oxidation results in decomposition products of little or no insecticidal activity. Conversion of phorate to activation or degradation products affects toxicity, and the rates of these changes determine the duration of its effectiveness after treatment.

Field and laboratory methods have been advanced to test the responses of insects and mites to toxic chemicals, but bioassays using phytophagous mites have been limited largely to contact applications (spraying or dipping mites anchored to plant material or held starved on adhesive tape, or exposing mites to treated surfaces). Assays based upon feeding mites on systemically treated plants will be most meaningful if there is previous knowledge of the relative toxicity of the metabolites. This must be coupled with measurement of the oxidative state of the systemic compound (or compounds) within the plant tissues used, and at least a standardization of the physiological condition of the plant, if repeatable results are to be obtained.

The broad objective of this study is the measurement of the relative

toxicity of phorate and phorate metabolites (made available in liquid feeding formulations) to the two-spotted spider mite, Tetranychus urticae Koch, and potato leafhopper, Empoasca fabae Harris. In the course of these investigations it has been necessary:

1. to develop a technique for confining the two-spotted spider mite and potato leafhopper that will afford ease of observation and recovery of test organisms;

2. to provide test organisms with feeding-access to liquid diet formulations (including toxicants) but without bodily contamination;

3. to standardize test mites, insects, and conditions for repeatable results;

4. to assay the toxicity of phorate-treated lima bean plants to the test mites.

REVIEW OF LITERATURE

Phorate and its Oxidative Metabolites

The oxidative metabolism of phorate yields five insecticidally active metabolites. Phorate and its metabolites, Figure 1, are inhibitors of cholinesterase and thereby are highly toxic to mammals (Bowman and Casida, 1957; Metcalf et al., 1957).

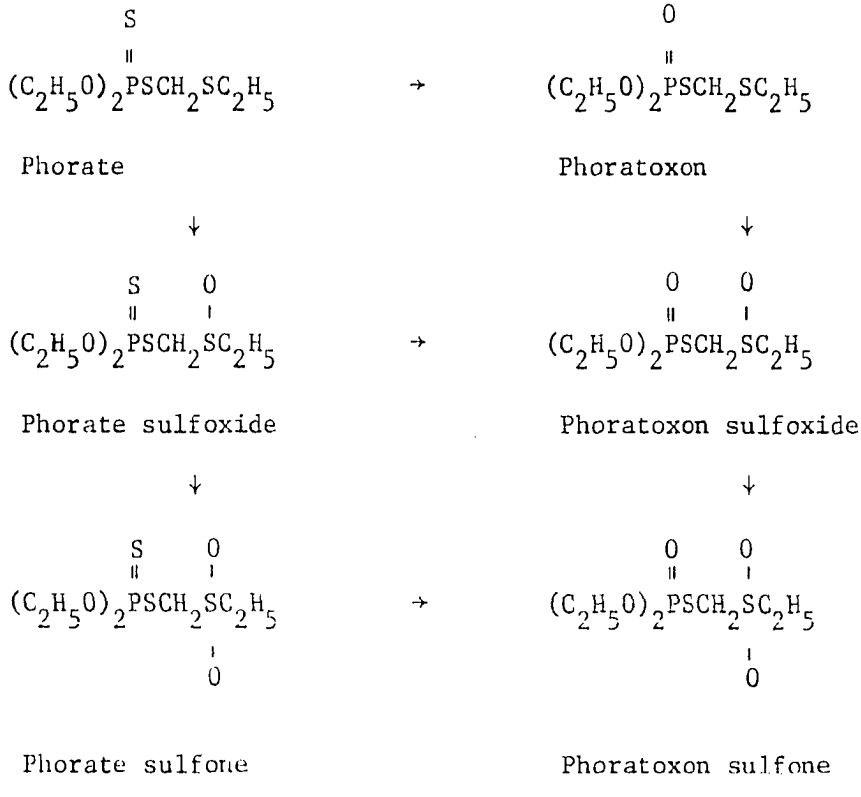


Figure 1. Oxidative metabolites of phorate

The oxidative metabolites are more potent cholinesterase inhibitors than phorate and are finally hydrolized to diethyl phosphoric acid and diethyl thiophosphoric acid; neither showed appreciable toxicity to insects (American Cyanamid Company, 1956).

Phorate oxygen analog sulfoxide and sulfone were formed in soil when phorate was added to sieved Canfield silt (Bache and Lisk, 1966). Metabolites similar to those formed in plants were also detectable in phorate-treated Sultan silt loam, and the possible conversion of the sulfoxide back to phorate in soil was postulated by Getzin and Shanks (1970). When tagged phorate was applied, 30% of the applied dose penetrated deeper than 3 inches within the soil and ca 95% remained unused by plants (Hacskaylo et al., 1961).

Relative to persistence of phorate per se in treated plants, phorate was detectable in some vegetables for 17 days following soil application and for 32 days after foliar spraying (Bowman and Casida, 1957), and phorate applied in foliar sprays to kale yielded eight different residual compounds over a period of two weeks. Only traces of the original material were detected within hours after treatment (Getz, 1962). Residues of phorate were found in plants and grains of wheat, oats, and barley 47 days following soil application (Lilly et al., 1958). In tomato fruits, phorate was found in concentrations of ca 0.1 and 4.0 ppm following soil and foliar applications, respectively (Van Middlelen, 1962). The presence of cholinesterase inhibitors was detectable 141 days following treatment in phorate-drenched potted chrysanthemum (Anderson et al., 1961).

Relative Toxicity of Phorate and Phorate Metabolites

Only few relative-toxicity evaluations of phorate metabolites are reported in the literature. Administered subcutaneously to male albino rats, phorate oxygen analog and its sulfone were the most toxic of the derivatives (24-hr LD_{50} of 0.6 to 0.8 mg/kg), followed in declining order

of toxicity by phoratoxon sulfoxide, phorate sulfone, and phorate sulfoxide. Phorate was the least toxic of all six compounds (24-hr LD_{50} of 8.0 to 10.0 mg/kg) and was a weak cholinesterase inhibitor (Bowman and Casida, 1957, 1958). However, in topical application to the abdomens of female house flies, Musca domestica L., Bowman and Casida (1957) reported that phorate was as toxic as the phoratoxon sulfone, while the latter was the most potent cholinesterase inhibitor of all the derivatives. The LD_{50} for both compounds was 1.5 mg/kg. The oxygen analog of phorate was the most toxic (LD_{50} of 1.1 mg/kg), while the sulfoxide compounds of phorate and its oxygen analog proved least toxic.

In toxicity evaluation of phorate and phorate metabolites, seven-day old larvae of the European corn borer, Ostrinia nubilalis Hübner, were maintained on artificial diet in plastic jelly cups covered with paperboard caps lined with saran. Dilutions of phorate and each of its metabolites were distributed on the diet surface before introducing the larvae. Data on 72-hr mortality indicated that phorate was more toxic than the derivatives, while the sulfones were least toxic. Phoratoxon sulfone, the most potent cholinesterase inhibitor, was not effective in killing European corn borer larvae within 72 hours (Bowman et al., 1969).

When crickets, Acheta pennsylvanicus Burmeister, were sprayed with a 10^{-3}% or 10^{-4}% concentration of phorate, it was considerably less toxic than its oxygen analog or the sulfone of the oxygen analog. However, no appreciable difference in toxicity of all six compounds was observed when applied at 10^{-2}% concentration. Added to the soil where crickets were kept for different periods of time, phorate was most toxic while sulfoxide or sulfone compounds of the phorate oxygen analog were the least toxic (Getzin and Shanks, 1970).

Mite Resistance to Phorate

The effectiveness of phorate against field infestations of the two-spotted spider mite has been variable. One reason for variable control was the development of inheritable mite-resistance to organophosphorous compounds. The elimination of susceptible mites in a population left only few tolerant individuals whose characteristics could be fixed by back crossing male offspring with the maternal parent. This resulted in the rapid development of resistance. Phorate was ineffective against parathion-resistant or aramite-resistant mites (Smith, 1952, 1960). While populations of the two-spotted spider mite develop varying degrees of organophosphorous resistance, very little is known about the biochemical mechanisms involved (Matsumura, 1964). Resistance to parathion and other organic compounds was observed as early as 1949 (Jefferson, 1956) and the possibility of the transmission of resistance between strains of the two-spotted spider mite was recognized (Taylor and Smith, 1956). On cotton, two-spotted spider mite infestations exhibited varying degrees of resistance to phorate applications (Mistric, 1964).

Because of the enormous reproductive potential of two-spotted mites and the population increases apparently associated with the use of chlorinated hydrocarbon insecticides (Klostermeyer, 1951; U.S. Dept. of Agric., 1958; Mistric, 1957; Oatman, 1960), acaricide resistance became a formidable problem to field crop, fruit, and vegetable growers and to greenhouse operators. It was estimated that in severe infestations by the two-spotted spider mite, over 100,000 acres of cotton sustained a 40% reduction in yield in the Southeastern United States (McGregor, 1928). The two-spotted spider mite has world-wide distribution (Ewing, 1914)

and 44 host plants in 24 families were recognized as early as 1893 by Harvey. Many more have been added since.

The Potato Leafhopper

Potato leafhopper, E. fabae Harris, is recognized as a major pest of potatoes, bean, and alfalfa. This leafhopper is capable of inflicting severe damage to host plants, reducing their vigor and yield. Heavy feeding causes characteristic hopperburn and leads to distortion and defoliation (Ball, 1919; DeLong, 1938; Putman, 1941). Kirollos (1969) found that fully developed nymphs of E. fabae failed to eclose from eggs in agar media containing as little as 1 ppm phorate, whereas an average of 70% eclosion occurred in phorate-free controls.

Methods of Evaluating Phorate Toxicity

Methods have been advanced to obtain toxicological data on relative efficacy of pesticides in the laboratory. Evaluation techniques currently used for insecticides or acaricides and described by Busvine (1957), Cleveland (1960), Ebeling (1960), Voss (1961), Lippold (1963) and Jeppson (1966) fall under one or more of these categories: topical application, exposure to treated plants, or exposure to chemical vapor. Siegler (1947) suggested the use of leaf disks (0.87 inch diam.) cut from infested plants and dipped in a test solution for evaluation of acaricides. The treated leaf disks were then maintained on moist cotton pads in petri dishes. The chemical quality of the residual acaricide was obviously uncontrollable and, with the use of a systemic compound, results would be affected by the physiological condition of the excised leaf disk, the degradative status of the test compound, and the structure of the leaf surface. And frequently there

has been no positive standardization of the number of mites or stage of their development. Nevertheless, Siegler's method has been modified and used by many workers.

Another assay method was introduced by Voss (1961) in which selected females were placed dorsal side down on adhesive tape. The adhesive tape was supported on 3 glass rods fixed to a glass microscopic slide. The edges of the adhesive tape, covered with paper, were clipped down to the glass slide. Mites received a contact exposure to the acaricide when the slide assembly was dipped for 5 seconds into the test solution. After removal, mites were kept starved on the adhesive tape until examination after 1 or 2 days.

Jeppson (1966) used a similar technique in which a double-stick Scotch tape, mounted near the end of a microscope slide, served to secure female mites when the dorsal spines were pressed onto the exposed surface of the adhesive. Mites were then sprayed by means of a Paasche "2 in 1" artist's airbrush.

No attempt had been reported for the feeding of phytophagous mites or leafhoppers on systemic insecticides or acaricides without the use of treated plants. However, a few techniques for controlled-diet feeding were used in nutritional studies. Many different natural and synthetic membranes have been employed for artificial feeding of certain Hemiptera. Fish swim-bladder and beef mesentery membranes were used successfully in feeding the sugar-beet leafhopper, Circulifer tenellus Baker, by Carter (1927, 1928). Storey (1932) also used beef mesentery membrane for the corn leafhopper, Cicadulina nubila Naude, and, after defatting, for feeding Empoasca solana DeLong (Herford, 1935). Insects were also fed on paraffin-covered

starch-agar media. A thin paraffin membrane, 60-120 microns, was employed for sugar-beet leafhopper feeding (Fife, 1932). Leafhopper, Orosius argentatus Evans, was fed through a plastic membrane (Day and McKinnon, 1951) or through a gauze (Nuorteva, 1951, 1952). Dahlman (1963) fed E. fabae through filter paper over an agar matrix impregnated with the nutrient solution. A membrane of either cabbage, iris, or tulip leaf epidermis was used to feed the peach aphid, Myzus persicae Sulzer, by Hamilton (1935), but Mittler and Dadd (1964) used Parafilm-M® for aphid feeding on various fluid diets.

In nutritional studies of the mite T. urticae, Rodriguez and Hampton (1966) confined females with a sticky barrier to a collodion film on a filter paper disc. The filter paper was placed inside a Gelman filter holder modified to hold a nutrient-soaked cotton pad and covered with a cover slip. The nutrient cotton pad was later replaced by a plastic sponge filled with nutrient solution (Rodriguez et al., 1967). Walling et al. (1968) used a Nalgene® centrifuge-tube (29 mm i.d.) cage that was closed at the top and bottom with polypropylene Nalgene tube-closures. By means of a sticky barrier of Stikem®, mites were restricted inside the cage to a resin membrane made of Butvar® B-76. The nutrient pad below the membrane was a sponge-like industrial synthetic foam.

EXPERIMENTATION

Materials and Techniques

Mite culture

A culture of the two-spotted spider mite, T. urticae Koch, was maintained on plants of lima bean, Phaseolus lunatus L., under a 14-hour light regime at 80±2°F and 65% relative humidity. The culture originated from mites collected from a neglected apple orchard in the vicinity of Ames, Iowa. Lima bean seeds were periodically planted in a steamed mixture of one part sand (by volume) to two parts of Webster clay loam, placed in perforated metal greenhouse flats. Each flat, resting in a shallow metal tray used for subwatering, was placed within a cage, 50 X 60 X 60 cm. Each cage was provided with a glass top and sliding glass front. The back and sides of the cage were covered with 100-mesh saran screen. Four culture-cages were held on overturned clay pots encircled inside and out with Tree Tanglefoot® (Tanglefoot Company, Grand Rapids, Michigan) to trap wandering mites. A flat of fresh plants was provided to replace depleted plants when necessary, and a number of mite-infested plants were placed among fresh plants to re-establish the infestation.

Standardization of mites for experiments

With environmental and nutritional factors held relatively constant throughout the experiments, uniform age of mites for the tests was desired.

Only adult females of T. urticae a few days old were included in the bioassays. To obtain this age-group of mites, 40-50 adult females were placed for oviposition on each of 12 detached lima bean leaves. Each

detached leaf was placed ventral side up on a wet cotton pad within a 70 X 95 X 22 mm. plastic snap-box. Excess water drained off through a hole at one side of the box. A hole at the opposite side of the box provided ventilation. Twelve snap-boxes were held vertically 20 mm. apart in a wooden holder (Figure 2a).

Mites were allowed 24 hours for egg laying and then removed to fresh oviposition cages. Eggs that hatched were kept, while the remainder were destroyed. Of those that hatched, one group of mites was held for observation of development, while the others of the same age-group were transferred to potted lima bean plants in isolated cages. When mites in the observation cage reached the adult stage, the corresponding group on lima bean plants was considered suitable for test. Mites were handled with a no. 000 sable brush.

Leafhopper culture

Test leafhoppers were obtained from a culture of the potato leafhopper, E. fabae, maintained on broadbean, Vicia fabae L., plants in the Iowa State University insectary greenhouse, in screen cages 50 X 60 X 60 cm. under natural daylight regime at 70-80°F and variable uncontrolled relative humidity. The culture was renewed annually from natural infestations on alfalfa.

Standardization of leafhoppers for experiments

In selecting leafhoppers of uniform age for bioassays, two-week-old seedlings of broadbean were infested by introducing 50 adults (both males and females) collected from the culture cages with the aid of an aspirator. Four weeks after infestation most nymphs had developed into adults and

were ready to use in tests within a few days of emergence. Over 600 adult leafhoppers could be collected by aspiration from a successfully reproducing culture within one cage.

Confinement and feeding on liquid formulations

A feeding cell was designed to confine two-spotted spider mites and to offer access to liquid formulations of selected chemicals. The feeding cell was comprised of acrylic tubing 2 mm. thick, and 23 mm. o. d. (Plexiglass®; Rohm and Haas Company, Philadelphia). The tubing was cut into sections 16 mm. long. Cut surfaces were ground smooth. Four holes, 6.5 mm. diam., were cut through the tubing at equally spaced intervals (Figure 2c). A standard paper punch was used to cut brass screen discs (100 mesh; .102 mm. diam. wire; .15 mm. aperture) that fit within each 6.5 mm. hole. Duco® cement (E. I. du Pont de Nemours Co., Inc., Wilmington, Delaware) liquified in ethanol was used to fill any gap around the disc and to secure it in place.

To one open end of the tubing section, a piece of Parafilm (Marathon Division, American Can Company, Neenah, Wisconsin) ca. 12.5 X 12.5 mm. was stretched and pressed down around the edge. It was further stretched by being pressed with a spatula into the section cavity to form a depression which later was to contain the liquid feeding formula. After the food droplet was in place, a second piece of Parafilm, ca. 25 X 25 mm. was placed over the droplet and pressed over and around the edge of the tubing, completely enclosing the feeding pocket. When pressed properly, the feeding cell could be overturned with no leakage of the solution within.

When the desired number of mites or leafhoppers had been introduced

through the open end of the cell, it was closed with a 22 mm. circular-glass cover slip (Figure 2c). Two strips of transparent tape, 8 mm. long and 3-4 mm. wide, secured the cover slip.

The mites on test diets could be observed through the glass cover as well as through the acrylic walls of the feeding cell under a binocular dissecting microscope. Only low magnification was necessary for observing the leafhoppers.

Confinement of mites to intact leaves

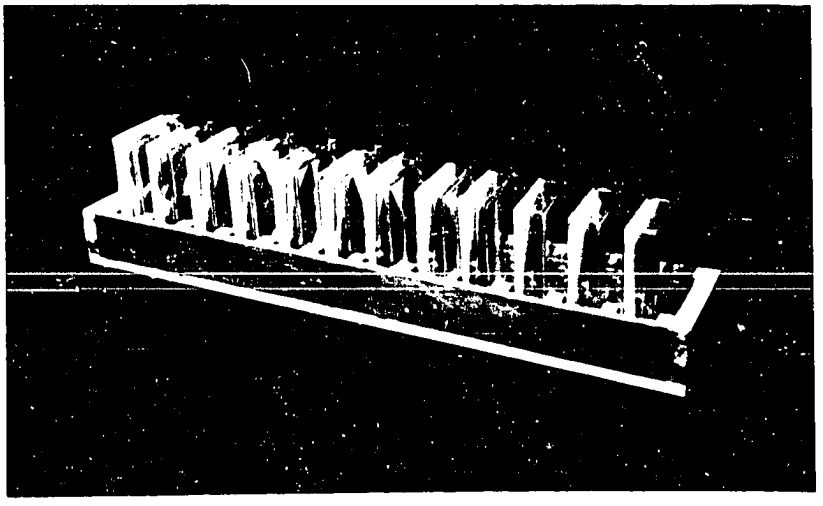
An alternative use of the feeding cell was to cement it to the leaf surface by applying Weldit® cement (Weldit Corporation, New York, N.Y.) to the rim of the cell and securing it to a leaf surface. The Parafilm was omitted and the cover-glass was used to close the open end of the cell. Leaves holding such feeding cells were supported on wooden racks (Figure 2b). A long-balance binocular dissecting microscope was used to aid in observing the mites within the feeding cells. This method was used to prevent the loss of mites, often activated by the repellent quality of the insecticide, especially evident at high rates of concentration. In preliminary experiments, Tree Tanglefoot®, vaseline, or lanolin applied as a ring around the petiole or on plant leaf surface, was unsatisfactory. For example, of 300 adult females transferred, 10 per leaf, onto phorate-treated lima bean seedlings, a considerable portion (40%) was lost within 24 hours. While some mites were observed caught in the barrier ring, others presumably dropped off the plant. Clip-on cages made similar to those described by Anderson et al. (1961) also allowed many mites to escape.

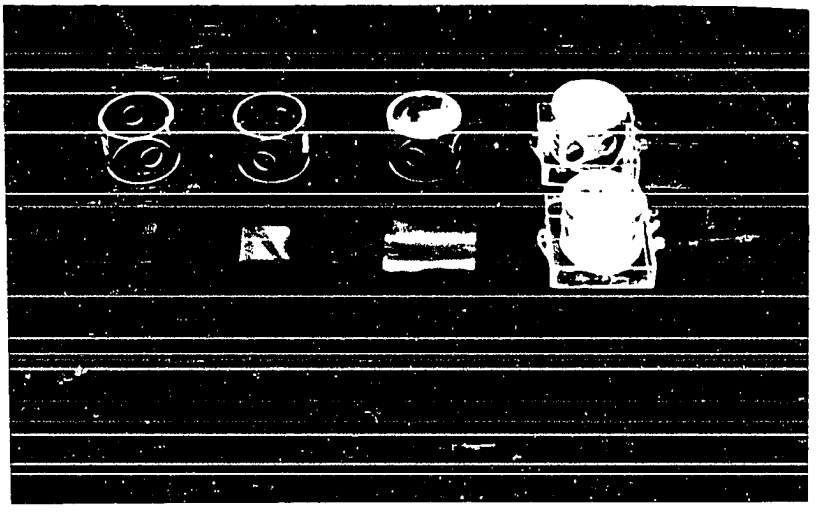
Figure 2. Devices for the confinement and feeding of T. urticae

a. Oviposition cages with cotton pad and leaf (ventral surface exposed) for standardizing age of test mites

b. Microcage for confining mites to lima bean seedlings and supportive wooden racks

c. Parts and progressive assembly of liquid formulation feeding cell





Survival on Liquid Diets

Survival of mites

Survival of the two-spotted spider mite, T. urticae, on sucrose solutions was tested by using the described feeding cells. Five hundred adult females of the same age-group were equally and randomly distributed among 5 treatments. To each treatment 100 mites were confined, 10 to each of 10 feeding cells. In one treatment, mites were left without food throughout the test. A second group was provided water, while three other groups were provided sucrose solutions at 1.5, 3, or 6% concentration, respectively. Mortality counts were taken daily for 2 weeks. The number of eggs deposited was recorded daily for 5 days, and the fecal pellets were counted on the 7th day.

Mites tested fed upon the fluid diets through the parafilm membrane. Both water and sucrose solutions increased survival time in comparison with survival time of adult females held without water or nutriment (Figure 3a; Appendix Table 6). Water alone extended 50% survival time (ST_{50}) by 1.3 day beyond that of starved ones. The addition of sucrose greatly extended survival time. The estimated ST_{50} values were 8.6, 9.6, and 12.1 day for 6, 3, and 1.5% sucrose solutions, respectively. In addition to its nutritional value, the presence of sucrose seemed a feeding arrestant.

The average number of fecal pellets was notably smaller in the absence of sugar (Table 1), presumably an evidence of but little imbibition. But, with higher sugar concentrations, sugar ingestion increased; this in turn increased the elimination of fecal pellets from the gut.

Although oviposition rate was not affected during the first 24 hours of

Table 1. Average number of T. urticae eggs and fecal pellets per feeding cell (10 females/cell)

Treatment	Day 1	2	3	4	5	Fecal pellets /1 cell
Starved	17.4	0.8	0.2	0.1	0.0	17.1
Water	17.0	2.4	0.9	0.7	0.6	36.9
1.5% Sucrose	17.3	3.7	3.5	2.2	0.3	57.1
3.0% Sucrose	18.5	4.3	3.3	1.4	0.0	50.8
6.0% Sucrose	20.8	2.8	2.3	1.9	0.4	57.8

confinement, it sharply declined thereafter regardless of the sucrose concentration provided (Table 1).

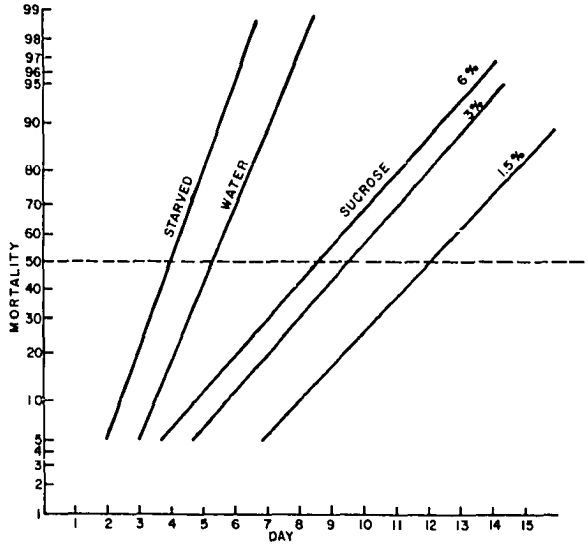
The number of adult female mites surviving 24 hours with access to phorate in 1.5% sucrose solutions was recorded. Phorate concentrations of 10, 25, 50, and 100 ppm were tested. For each test and a control (provided only with 1.5% sucrose solution) 10 adult females of the same age-group were confined in each of 10 feeding cells.

Data and probit analysis (Finney, 1952) on the toxic effects of phorate fed with sucrose are presented in Figure 3b and Appendix Table 7. At the highest level of toxicant tested, 100 ppm, mortality and/or symptoms of intoxication were rapid, occurring 12 hours following introduction of mites. Only 7% of mites were affected at 10 ppm after 24 hours. For the linear regression of mortality on dosage (Figure 3b), an insignificant X^2 value was calculated at 95% probability after correction for natural mortality by Abbott's formula (Abbott, 1925). The estimated LC_{50} occurred at 30.9 ppm

Figure 3. Mortality of T. urticae on liquid diets

a. Mortality curves for adult females, starved, with access to water, and to 6, 3 and 1.5% sucrose solution

b. Probit regression for mortality of adult females fed on phorate in 1.5% sucrose solution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DAY
STARVED
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SUCROSE
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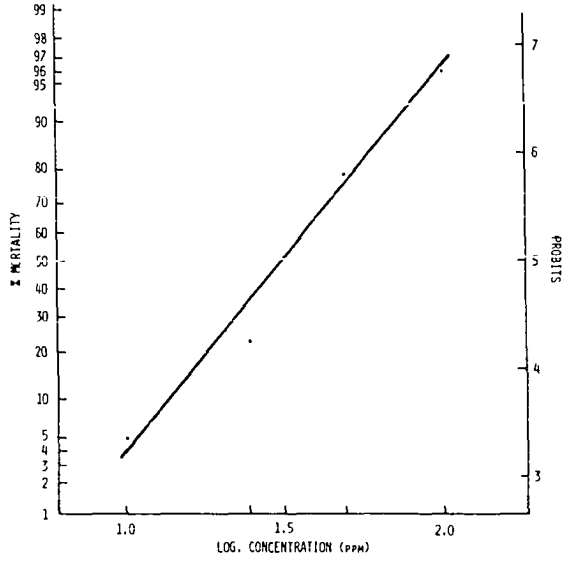
1.5%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LOG. CONCENTRATION (PPM)

phorate concentration. Coefficients of variability among replicate feeding-cells were 11.8, 11.1, and 7.3% for 10, 50, and 100 ppm concentrations respectively, compared with 21.1% for the control treatment. However, a higher coefficient of variability (65.7%) was obtained at 25 ppm.

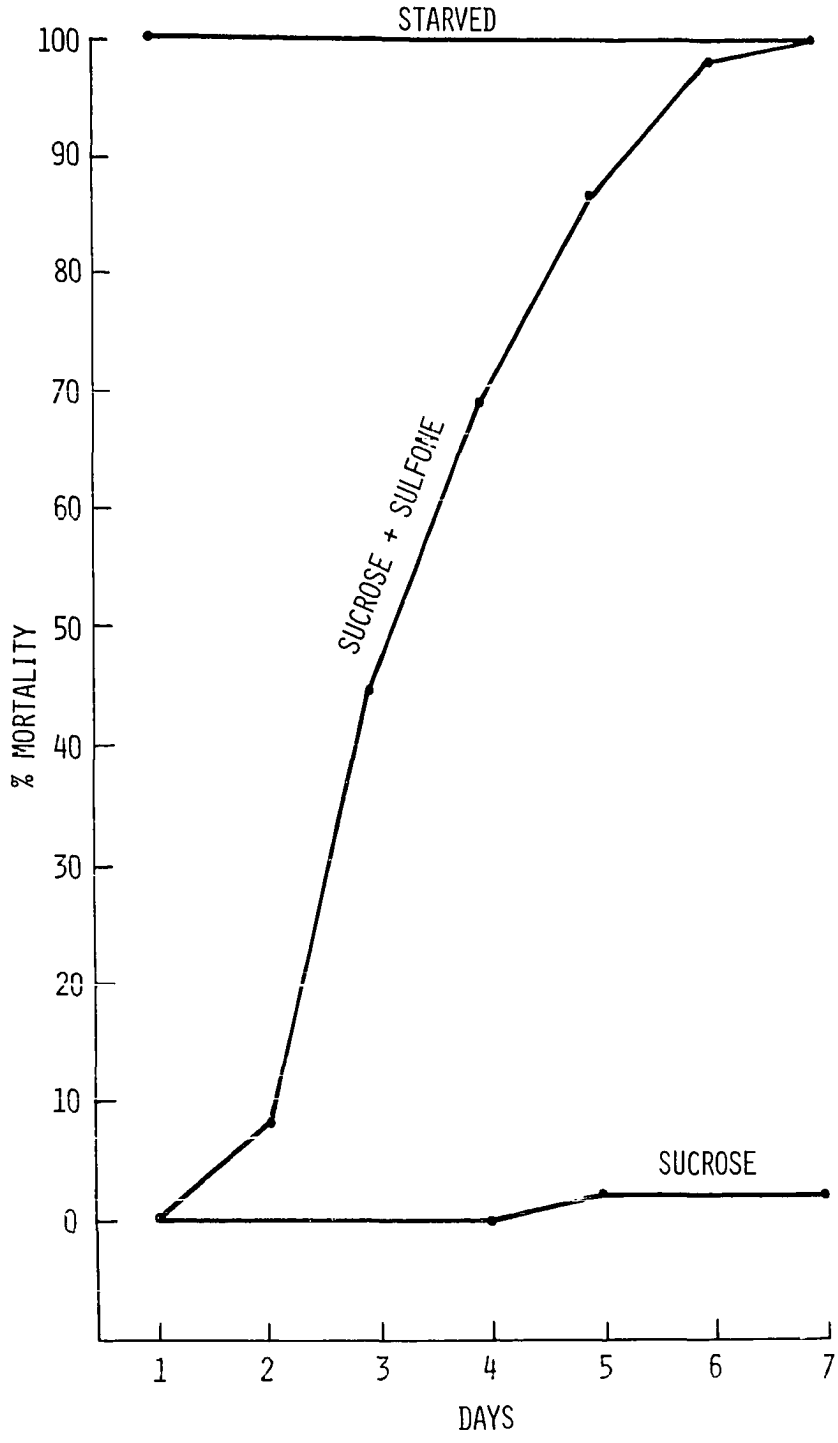
Toxic effects of phorate provided additional evidence of feeding intake. Toxicity symptoms included tremors (especially obvious was trembling of the legs) that continued while mites were anchored to webbing or to the inside wall of the feeding-cell. At death, the legs became ventrally involuted.

Survival of leafhoppers

Potato leafhopper feeding through the parafilm membrane and survival in feeding-cells were tested. Adult leafhoppers of the same age-group were aspirated and placed 5 into each of 30 feeding-cells, equally divided among 3 treatments. In one treatment, leafhoppers were kept starved. Leafhoppers in the other two treatments were provided either 1.5% sucrose or 0.08 ppm phorate oxygen-analog sulfone added to 1.5% sucrose solution. Observations of mortality were made daily for 7 days. However, mortality counts were discontinued after 24 hours in one treatment group (starved) since none of leafhoppers remained alive at this time.

Leafhoppers confined to feeding-cells resumed activities but no oviposition was observed. In the absence of toxic material 100% survival continued for 4 days with artificial feeding and only 2% mortality occurred after 7 days. The sulfone compound added was effective within 2 days of its ingestion and none of the leafhoppers survived on such diets beyond 7 days (Figure 4; Appendix Table 8). That the leafhoppers had imbibed of the

Figure 4. Mortality of E. fabae adults starved, with access to 1.5% sucrose solution, or 0.08 ppm phoratoxon sulfone in sucrose solution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liquid diet through the parafilm membrane of the cells used in this study was evidenced by extended survival time on sucrose diets, and the gradual effect of the systemic insecticide.

Toxicity Evaluation of Phorate and Five Phorate Oxidative Metabolites

Phorate and five oxidative metabolites, usually formed in plants (Figure 1), were each diluted to 1000 ppm by dissolving 10 mg of standard compounds in ethanol, adding 0.5 ml Triton X-100, and bringing to 10 ml in separate volumetric flasks. Only 5% ethanol was used for dilution when toxicant concentration in the diet for mites exceeded 100 ppm. From each solution dilutions were made in 1.5% sucrose for feeding. Chemicals were kept under refrigeration until needed for feeding experiments. A control dilution was included at each rate of concentration used, and it contained the corresponding amount of ethanol and Triton fraction in 1.5% sucrose but no insecticide.

Relative toxicity to mites

Concentrations tested for relative toxicity to mites included 6.25, 12.5, 25.0, 50.0, and 100 ppm of phorate. Phorate sulfoxide and sulfone each were tested at 50.0, 100.0, 200.0, 400.0, and 800.0 ppm concentrations. Phorate oxygen analog concentrations were used at the same rate as phorate except for the omission of 6.25 ppm and the addition of 200.0 ppm concentrations. The sulfoxide and sulfone compounds of the phorate oxygen analog each were tested at 25.0, 50.0, 100.0, 200.0, and 400.0 ppm concentrations.

For each test, 100 adult female T. urticae of the same age-group were placed, after a 3-hr starvation, 10 into each of 10 feeding cells.

Individual females were placed on the inner surface of the glass cover of the cell and left to move up the inverted feeding cell. Those that failed to move were replaced. Mortality counts were recorded 2 days after introducing the mites (Appendix Tables 9 and 10).

Evaluation of dosage-mortality curves was based on a probit analysis (Finney, 1952). Statistical data are presented in Table 2 and probit regression lines in Figure 5. Phorate was the most toxic of all six compounds with LC_{50} of 16.4 ppm (10 times greater than phoratoxon, the most oxidized metabolite) and ca 3 times more toxic than its oxygen analog. The sulfoxide derivative of phorate was the least toxic to the two-spotted spider mite, having an LC_{50} of 403 ppm. Phorate oxygen analog was still more toxic than its sulfoxide or sulfone compound (Figure 5b).

Table 2. Probit analysis of dose-mortality data from feeding phorate and its metabolites to T. urticae

Compound	$Y = \bar{y} + b\ (X - \bar{x})$ †	X^2	(v)b ††	LC_{50} ppm
Phorate	0.619 + 3.608 X	5.827 n.s.	0.0740	16.4
Phorate sulfoxide	-1.017 + 2.309 X	6.524 *	0.0636	403.6
Phorate sulfone	0.938 + 1.589 X	10.362 *	0.0245	359.7
Phoratoxon	1.230 + 2.218 X	7.960 *	0.0351	50.1
Phoratoxon sulfoxide	1.619 + 1.579 X	14.182 **	0.0281	138.4
Phoratoxon sulfone	0.823 + 1.903 X	5.696 n.s.	0.0293	156.7

† Y, mortality; $\bar{y}$, sample mean; b, regression coefficient; X, dosage; $\bar{x}$, mean dosage.

†† (v)b, variance of regression coefficient.

n.s. Not significant at 95% probability.

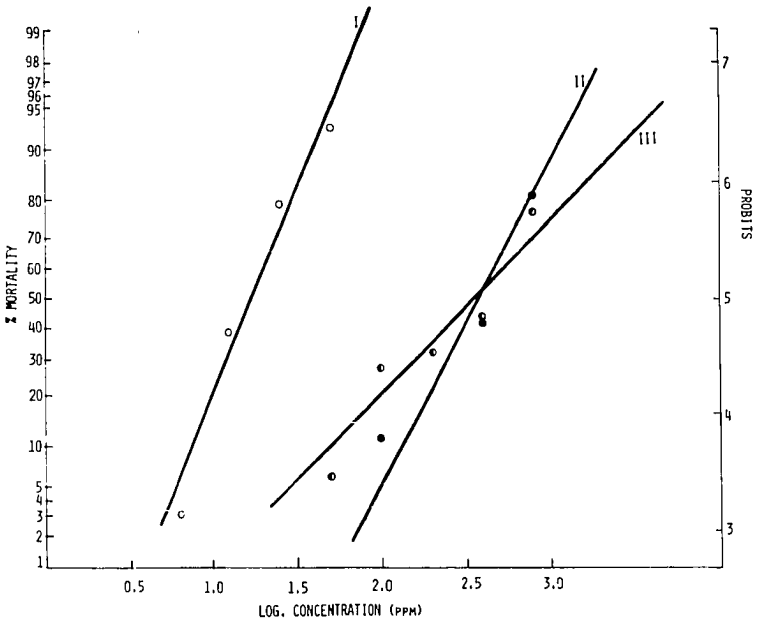
* Significant at 95% probability but not significant at 99%.

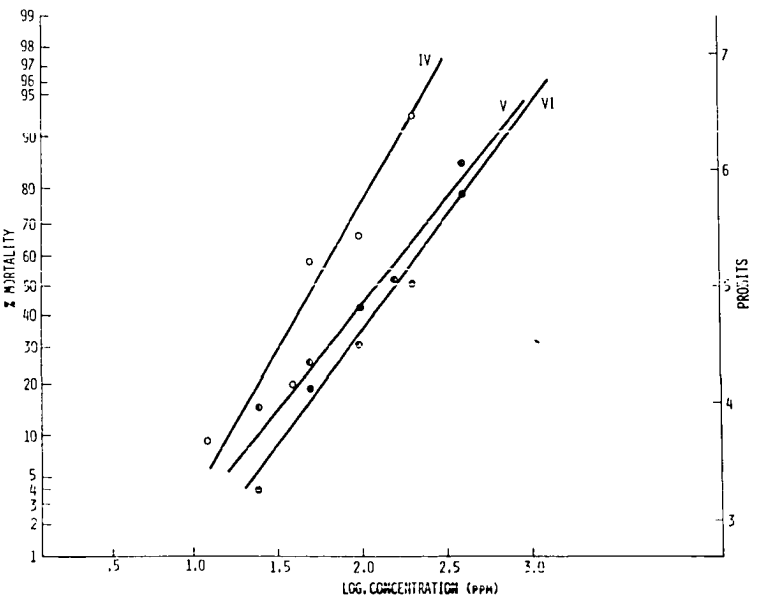
** Significant at 99% probability.

Figure 5. Mortality of T. urticae on phorate and phorate metabolites in 1.5% sucrose

a. Dosage-mortality curves for adult females with access to phorate (I), phorate sulfoxide (II), or phorate sulfone (III)

b. Dosage-mortality curves for adult females with access to phoratoxon (IV), phoratoxon sulfoxide (V), or phoratoxon sulfone (VI)


I
II
III
% MORTALITY
PROBITS
LOG. CONCENTRATION (PPM)


IV
V
VI
% MORTALITY
PROBITS
LOG. CONCENTRATION (PPM)

Relative toxicity to leafhoppers

One hundred adult leafhoppers of the same age-group were placed 10 into each of 10 feeding cells, and observed for possible injuries. Injured leafhoppers were replaced. Concentrations of phorate and each of its metabolites fed in 1.5% sucrose included, 0.016, 0.08, 0.1, 0.2, 0.3, 0.4, 1.0, 2.0, 5.0, and 10.0 ppm. An additional concentration of 1.5 ppm was tested for phorate only. Mortality counts were recorded 2 days after introducing the leafhoppers (Appendix Tables 11 and 12).

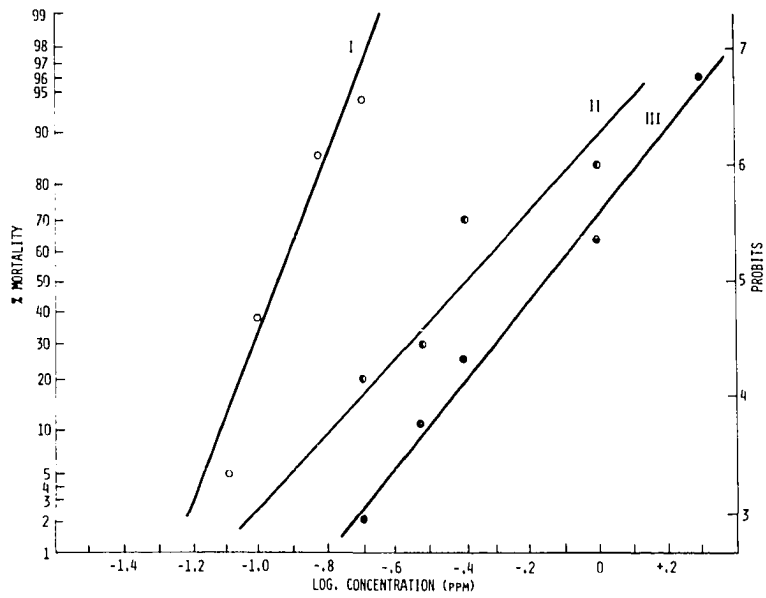
At the 0.08 ppm concentration, observation continued daily for 8 days and included adult leafhoppers (5 in each of 10 feeding cells) fed on each of the six test compounds and a control (Appendix Table 13).

Evaluation of dosage-mortality curves was based on a probit analysis (Finney, 1952). However, extremely low or extremely high mortality concentrations were excluded and 4 or 5 middle-range rates of concentration were sufficient for the analysis. Statistical analysis of the data (Table 3) showed that phorate in leafhopper diet was more toxic (LC_{50} of 0.12 ppm) than its oxidation products sulfoxide or sulfone (LC_{50} of 0.37 and 0.62 ppm respectively). The sulfoxide and sulfone compounds seemed to produce parallel probit regressions against their log. concentrations (Figure 6a) which suggested a constant difference between dosages producing similar mortalities, and consequently a constant relative potency at all levels of response. Phorate did not follow this pattern and produced a higher regression coefficient within a small range of concentrations. However, phorate oxygen analog was less toxic (LC_{50} of 0.50 ppm) than its oxidation products, sulfoxide or sulfone (LC_{50} of 0.10 and 0.16 ppm respectively), and near parallel regression lines were estimated (Figure 6b). None were

Figure 6. Mortality of E. fabae on phorate and phorate metabolites in 1.5% sucrose

a. Dosage-mortality curves for adults with access to phorate (I), phorate sulfoxide (II), or phorate sulfone (III)

b. Dosage-mortality curves for adults with access to phoratoxon (IV), phoratoxon sulfoxide (V), or phoratoxon sulfone (VI)


% MORTALITY
PROBITS
LOG. CONCENTRATION (PPM)
I
II
III

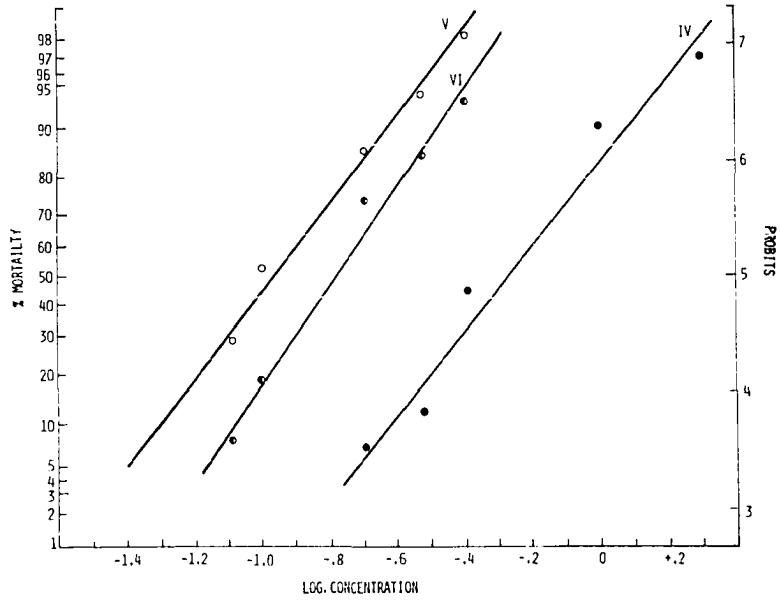

% MORTAILTY
PROBITS
LOG. CONCENTRATION
V
VI
IV

Table 3. Probit analysis of dose-mortality data from feeding phorate and its metabolites to E. fabae

Compound	$Y = \bar{y} + b\ (X - \bar{x})^{\dagger}$	X^2	$(v)b^{\dagger\dagger}$	LC_{50} ppm
Phorate	-3.310 + 7.812 X	7.58^{**}	0.3730	0.116
Phorate sulfoxide	0.525 + 2.846 X	17.73^{**}	0.0926	0.373
Phorate sulfone	2.867 + 2.696 X	8.62^{*}	0.0760	0.618
Phoratoxon	2.388 + 3.746 X	11.37^{**}	0.0639	0.498
Phoratoxon sulfoxide	1.284 + 3.660 X	$2.91^{n.s.}$	0.0930	0.104
Phoratoxon sulfone	-0.093 + 4.226 X	$4.96^{n.s.}$	0.0818	0.160

†Y, mortality; $\bar{y}$, sample mean; b, regression coefficient; X, dosage; $\bar{x}$, mean dosage.

††(v)b, variance of regression coefficient.

$^{n.s.}$Not significant at 95% probability.

*Significant at 95% probability but not significant at 99%.

**Significant at 99% probability.

Table 4. Cumulative % mortality of E. fabae during 8 days on each of phorate and phorate metabolites (0.08 ppm in 1.5% sucrose solution)

Compound	Day observed							
	1	2	3	4	5	6	7	8
Phorate	0	5	18	28	36	54	88	94
Phorate sulfoxide	0	0	8	14	40	58	70	86
Phorate sulfone	0	0	0	0	2	2	2	4
Phoratoxon	0	0	0	0	0	2	2	2
Phoratoxon sulfoxide	0	52	92	98	100			
Phoratoxon sulfone	0	8	44	70	84	98	100	
Control (1.5% sucrose)	0	0	0	0	0	0	0	0

parallel to the phorate dose-mortality curve. At 0.08 ppm concentration phorate was less effective within 8 days than the sulfoxide or the sulfone of its oxygen analog (Table 4; Appendix Table 13). The phorate oxygen analog at 0.08 ppm concentration was not effective within 8 days in killing adult leafhoppers, and neither was the phorate sulfone.

Phorate toxicity to mites on treated lima bean plants

The systemic effect of phorate on the two-spotted spider mite was tested by offering treated Henderson Bush lima bean seedlings as food. Seedlings were limited to their primary leaves. In preparation for treatment the roots were freed from soil and carefully washed, freed of surface water and then submerged in a chosen concentration of phorate (emulsifiable Thimet® concentrate, American Cyanamid Company, Agricultural Division, Princeton, N.J.) for a selected period of time. At the end of the immersion period, the insecticide was washed off the root surface and seedlings were replanted individually in a steamed soil mixture placed in 7 oz. insulated cups which had been provided with a hole on the bottom. Five seedlings provided for the treatment of 100 adult female mites of the same age-group, each seedling received 4 confinement cages, 2 per leaf, and each cage contained 5 adult females. Different treatments were held on separate units (Figure 2b).

Concentrations used for the bioassay were 200, 300, 400, 500, and 600 ppm of phorate, for each of 3- and 6-hour root-submergence treatments. Mortality counts were recorded daily for 7 days after introducing the mites (Table 5; Appendix Tables 14 and 15).

Variance analysis and paired comparisons (Appendix Table 16) for

Table 5. Cumulative % mortality of T. urticae during 7 days on phorate root-treated lima bean seedlings

Concentration (ppm)	Day observed						
	1	2	3	4	5	6	7
	Six-hr root-submergence						
200	12	41	68	73	78	83	87
300	16	40	65	78	85	88	91
400	11	42	65	83	87	92	93
500	23	47	73	88	95	100	100
600	21	51	72	87	96	98	98
	Three-hr root-submergence						
200	11	21	33	44	49	57	61
300	12	20	32	47	58	66	72
400	8	23	38	50	61	70	81
500	8	22	44	59	78	92	95
600	9	18	39	51	62	75	84
	Control						
0	0	2	5	7	9	9	11

significant differences at 95% of probability showed no appreciable differences in mortality within 24 hours regardless of the concentration of insecticide used or time of root exposure. The only exception was a significant difference between the untreated plants and any of the treated combinations. Treatment of 500 ppm for 6 hours caused mortality exceptionally higher than 600 ppm root treatment for 6 hours and consequently gave significant mortality differences over all concentrations where roots were submerged for 3 hours. The difference in percentage mortality between the

untreated control and any other combination of phorate treatment remained significant throughout the test. No appreciable difference occurred at the end of 7 days between mortality from application of 200, 300, or 400 ppm phorate for 6-hour root-dip.

But, generally, 3-hour root submergence produced less effect on mite mortality than 6-hour treatment. Mortality reached its peak within 2 days of mite exposure to 6-hour treated plants. Three days were required for maximum daily mortality among mites on 3-hour-treated plants (Table 5).

In a second treated-plant experiment, the roots of lima bean seedlings were submerged for 3 hours in 500 ppm phorate. One group of 100 adult female mites were confined, in feeding cages, to leaves of the treated seedlings for 8 days. Eight additional groups of 100 mites each were allowed to feed successively, one group at a time, for only 24 hours on the treated seedlings then removed (10 to each of 10 untreated detached leaves kept on moist cotton pads (Figure 2a)) and held 20 days of daily mortality counts (Appendix Table 17). When mortality of the mites exposed continuously was compared with that of the groups exposed in successive 24-hour periods, the mortality of the former group was greatest. There was slightly greater mortality among mites introduced to treated leaves 24-hours after root treatment than among groups of mites introduced in subsequent 24-hour periods.

Absorption of phorate through lima bean roots took place within 3 hours of root submergence in emulsifiable liquid formulation and produced intoxication of adult female two-spotted spider mites within 24 hours of their exposure to treated plants. The higher the concentration used, within the test range, the more pronounced was the toxic effect. However,

at 500 ppm and higher, phytotoxic symptoms were observed including "leaf-burn" spots. Seedlings were not kept long enough for observation of possible phytotoxicity at lower rates of treatment.

Continuous vs 24-hour mite exposure in successive groups, 1-day apart after treatment, did not indicate any enhancement of the toxic effect of phorate present in lima bean leaf tissues, since percentage of mites killed did not appreciably increase with lapse of time.

SUMMARY AND CONCLUSIONS

Phorate and five oxidative metabolites of phorate in liquid diets containing sucrose were assayed for toxicity to the two-spotted spider mite, T. urticae, and the potato leafhopper, E. fabae. Sucrose was added to liquid formulations of the systemic compounds to stimulate feeding.

Phorate was more toxic to the two-spotted spider mite than any of its oxidative metabolites, and the phorate oxygen analog was relatively more toxic to mites than its sulfoxide or sulfone metabolites.

Phorate and its sulfoxide and sulfone compounds were equally toxic to potato leafhoppers. In declining order of toxicity, phorate was followed by the sulfoxide, the phorate oxygen analog, and the phorate sulfone.

Based on LC_{50} estimates, phorate and phorate metabolites were more highly toxic to the potato leafhopper than to the two-spotted spider mite. However, phorate per se was more toxic to both organisms than were phorate oxidative metabolites.

Evidence was lacking to support the hypothesis that the phytosystemic organophosphorous phorate exerted its toxic action in the inhibition of cholinesterase systems of either the two-spotted spider mite or the potato leafhopper inasmuch as there was no consistent correlation between the oxidative state of phorate metabolites and their toxic effectiveness. The hypothesis was based on work with mammals and in vitro evidence of increased cholinesterase inhibition with advanced oxidation of phorate. Further, the functional elements for the hypothesis (the presence of acetylcholine, acetylcholinesterase, and choline, and the release of acetylcholine on stimulation of nerve endings) are present in insects (Smallman, 1956),

and there is positive evidence of the development of the cholinergic system during late embryonic stages in insects (Smith, 1956).

Despite reports in the literature that oxidation of phorate enhances cholinesterase inhibition (Bowman and Casida, 1957), oxidized phorate (phorate oxygen analog) was less toxic than phorate to the two-spotted spider mites and potato leafhoppers, and phorate (a weak inhibitor of cholinesterase) was generally more toxic than the more potent cholinesterase-inhibiting metabolites.

When mites were introduced at intervals, 1 day apart, after lima bean root-treatment, no enhancement of toxicity occurred on any of the successive eight days of exposure. Eight days would bracket the period when the oxidation of phorate would be expected to occur in plant tissues on the basis of time relationships in the oxidation of phorate in various plants reported by Metcalf et al. (1957).

The primary leaves of lima bean seedlings following 3 hours of root submergence were toxic to two-spotted spider mites. No significant increase of toxicity to two-spotted spider mites resulted within 24 hours from 6-hour submergence of roots in phorate. However, a significant increase in mortality of continuously exposed mites resulted at a later date following the 6-hour root submergence. This mortality increase persisted at a significant level throughout 7 days.

The method described offered a new facile means of sustaining the two-spotted spider mite, T. urticae, on liquid diet and was useful for testing acaricides in liquid formulations directly through ingestion. The method could be used for other phytophagous mites or insects and has proved useful for potato leafhopper, E. fabae, providing ease of observation, use

of small amount of liquid test chemical (0.5 ml or less) and recovery of all organisms tested.

The feeding-cell could be positioned for upside down or right-side up feeding.

Evidence of feeding through the Parafilm membrane in the feeding-cells included:

1. An extended survival time of both adult female two-spotted spider mites and adult potato leafhoppers which were confined to water and/or sucrose solutions.

2. Increased ingestion suggested by an increase in the number of fecal pellets during periods of access to sucrose.

3. The observation of red-dyed food in the gut of leafhoppers and mites that had access to sucrose-rhodamine B diet.

4. The appearance of intoxication symptoms and death following access to phorate and/or the phorate oxygen analog sulfone.

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The contribution of phorate and phorate metabolites from American Cyanamid Company is gratefully acknowledged.

APPENDIX

Table 6 . Number of adult female T. urticae surviving during 15 days on liquid diet (10 adults/cell)

Replicate	Day observed														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Starved														
I	10	9	9	7	3	0									
II	10	10	8	5	4	1	0								
III	10	8	7	6	5	1	0								
IV	10	10	10	8	5	0									
V	9	9	9	5	0										
VI	10	10	10	7	0										
VII	10	10	10	7	0										
VIII	10	9	8	6	1	0									
IX	10	10	10	3	0										
X	10	9	6	2	0										
%	99	94	87	56	18	2	0								
	Water only														
I	10	10	10	9	8	0									
II	10	10	10	10	6	2	0								
III	10	10	10	10	7	2	1	0							
IV	10	10	10	9	5	0									
V	9	9	9	9	8	2	2	0							
VI	10	10	10	9	6	3	3	1	0						
VII	10	10	10	9	6	2	1	0							
VIII	10	10	10	10	7	2	2	0							
IX	10	10	10	10	9	5	1	0							
X	10	9	9	7	5	2	0								
%	99	98	98	92	68	20	10	1	0						
	1.5% sucrose														
I	10	10	10	10	10	10	10	10	10	6	3	1	0		
II	10	10	10	10	10	10	10	10	10	10	10	7	6	6	5
III	10	10	10	10	10	10	10	10	10	7	5	4	4	2	2
IV	10	10	10	10	10	10	9	9	9	8	8	8	7	6	6
V	10	10	10	10	10	10	10	10	10	10	10	9	7	6	4
VI	10	10	10	10	10	10	10	10	9	6	5	4	2	1	0
VII	10	10	10	10	10	10	10	10	8	8	8	8	3	3	3
VIII	10	10	10	9	9	9	9	9	7	5	5	2	2	2	1
IX	10	10	10	10	10	10	10	8	7	5	4	2	0		
X	10	10	10	9	9	9	9	8	6	5	3	1	1	0	
%	100	100	100	98	98	98	97	94	86	70	61	46	32	26	21

Table 6 . (Continued)

Replicate	Day observed														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	3.0% sucrose														
I	10	10	10	9	9	9	9	8	6	6	4	3	1	1	0
II	10	9	9	9	9	9	8	7	7	6	6	2	2	2	1
III	10	10	10	10	9	9	8	7	6	4	3	2	1	0	
IV	10	10	10	10	10	9	8	7	6	5	2	1	1	0	
V	10	10	10	10	9	9	8	8	6	5	4	3	0		
VI	10	10	10	10	10	10	9	7	7	6	3	2	1	1	0
VII	10	10	10	10	9	8	6	6	5	4	3	1	1	0	
VIII	10	10	10	10	10	10	8	7	6	5	4	0			
IX	10	10	10	10	9	8	8	7	5	4	1	1	1	0	
X	10	10	10	10	10	9	9	8	7	5	5	3	2	1	1
%	100	99	99	98	94	90	81	72	61	50	35	18	10	5	2
	6.0% sucrose														
I	10	10	10	10	10	10	9	8	8	6	4	3	3	2	0
II	10	10	10	10	8	8	8	8	7	5	5	5	3	3	1
III	10	10	10	10	10	7	5	5	5	4	2	2	0		
IV	10	10	10	10	9	8	7	6	5	5	3	1	1	0	
V	10	10	9	9	6	5	4	4	2	2	0				
VI	10	10	10	10	8	6	6	4	3	2	2	0			
VII	10	10	9	9	9	8	6	5	5	5	4	4	3	1	0
VIII	10	10	10	10	7	7	7	5	4	3	3	1	0		
IX	10	10	10	10	9	9	7	5	3	2	2	2	2	1	1
X	10	10	10	9	8	7	4	4	2	2	1	0			
%	100	100	98	97	84	75	63	54	46	36	28	18	12	7	2

Table 7. Number of adult female T. urticae surviving 24 hr on phorate in 1.5% sucrose (10 adults/cell)

Phorate combination	Replicate feeding cells										% Mortality at 24 hrs
	I	II	III	IV	V	VI	VII	VIII	IX	X	
0 ppm (1.15% sucrose)	10	10	10	9	10	10	10	10	9	10	2
10 ppm in (1.5% sucrose)	10	9	8	10	9	10	10	9	10	8	7
25 ppm in (1.5% sucrose)	8	9	6	7	8	5	8	10	9	6	24
50 ppm in (1.5% sucrose)	3	1	2	3	1	3	3	2	2	1	79
100 ppm in (1.5% sucrose)	0	0	0	1	0	0	0	2	1	0	96

Table 8 . Number of adult E. fabae surviving 1.5% sucrose, starvation, or 1.5% sucrose + 0.08 ppm phoratoxon sulfone (5 adults/cell)

Observation day	Replicate feeding cells									
	I	II	III	IV	V	VI	VII	VIII	IX	X
	1.5% sucrose									
1	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5
5	5	5	4	5	5	5	4	5	5	5
6	5	5	4	5	5	5	4	5	5	5
7	5	5	4	5	5	5	4	5	5	5
	Starved									
1	0	0	0	0	0	0	0	0	0	0
	1.5% sucrose + 0.08 ppm phoratoxon sulfone									
1	5	5	5	5	5	5	5	5	5	5
2	5	5	4	5	5	4	4	5	5	4
3	5	2	2	4	3	2	1	3	3	3
4	3	2	2	3	0	1	0	1	2	1
5	2	2	0	1	0	1	0	1	0	1
6	0	1	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0

Table 9. Number of adult female T. urticae dead within 48 hours of feeding on phorate and its sulfoxide and sulfone in 1.5% sucrose solution (10 adults/cell)

Concentration (ppm)	Replicate feeding cells										Mortality %
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Phorate											
6.25	0	1	1	0	0	0	1	0	0	1	4.0
12.50	5	3	3	2	5	4	3	7	2	4	38.0
25.00	10	6	9	7	8	7	9	6	8	9	79.0
50.00	10	8	10	10	9	9	10	9	10	8	93.0
100.00	10	10	10	10	10	10	10	10	10	10	100.0
Phorate sulfoxide											
50.00	0	0	0	0	0	0	0	0	0	0	0.0
100.00	0	1	0	2	1	1	2	1	2	1	11.0
200.00	2	3	3	2	1	2	1	3	2	2	21.0
400.00	5	7	4	7	3	3	3	4	3	5	44.0
800.00	10	6	9	9	10	9	8	8	7	6	82.0
Phorate sulfone											
50.00	1	0	2	0	0	3	0	0	0	0	6.0
100.00	2	0	1	1	3	5	5	3	5	2	27.0
200.00	5	5	2	4	1	3	4	5	2	1	32.0
400.00	7	3	6	6	5	2	2	4	5	5	45.0
800.00	5	8	7	9	10	8	7	7	9	8	78.0

Table 10. Number of adult female T. urticae dead within 48 hours of feeding on phoratoxon, phoratoxon sulfoxide, or phoratoxon sulfone in 1.5% sucrose (10 adults/cell)

Concentration (ppm)	Replicate feeding cells										Mortality %
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Phoratoxon											
12.50	1	1	0	1	3	0	0	2	0	1	9.0
25.00	3	2	0	4	2	2	3	0	1	3	20.0
50.00	6	5	7	7	8	5	4	5	5	7	59.0
100.00	6	5	7	8	6	8	6	7	8	7	68.0
200.00	10	9	10	9	9	10	8	9	9	10	93.0
Phoratoxon sulfoxide											
25.00	0	1	3	2	1	0	3	2	2	1	15.0
50.00	1	2	5	5	3	3	2	2	2	1	26.0
100.00	3	3	4	4	3	2	3	6	2	2	32.0
200.00	4	6	5	6	4	7	3	7	6	5	53.0
400.00	10	9	8	8	9	10	10	7	10	6	87.0
Phoratoxon sulfone											
25.00	1	0	1	0	0	0	1	0	1	0	4.0
50.00	2	2	1	1	2	1	3	3	2	2	19.0
100.00	6	3	3	2	4	6	6	4	5	5	44.0
200.00	6	6	4	5	5	5	5	4	5	6	51.0
400.00	7	8	7	8	6	9	8	9	8	10	80.0
Control											
6.25	0	0	0	0	0	1	0	0	0	0	1.0
12.50	0	0	0	0	0	0	0	0	0	0	0.0
25.00	0	0	0	0	0	0	0	0	0	0	0.0
50.00	0	0	0	0	0	0	0	0	0	0	0.0
100.00	0	0	0	0	1	0	0	0	1	0	2.0
200.00	0	0	0	0	0	0	0	0	0	0	0.0
400.00	1	0	0	1	0	1	0	1	0	0	4.0
800.00	0	0	2	0	0	0	0	2	0	0	4.0

Table 11. Number of adult E. fabae dead within 48 hours of feeding on phorate, phorate sulfoxide, or phorate sulfone in 1.5% sucrose (10 adults/cell)

Concentration (ppm)	Replicate feeding cells I	II	III	IV	V	VI	VII	VIII	IX	X	Mortality %
					Phorate						
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	0	0	0	0	1	1	0	1	0	2	5.0
0.1	3	4	6	7	6	2	0	3	4	2	37.0
0.15	9	10	10	10	7	7	9	10	7	6	85.0
0.2	9	10	10	10	8	9	10	8	10	10	94.0
0.3	10	10	10	10	10	10	10	10	10	10	100.0
0.4	10	10	10	10	10	10	10	10	10	10	100.0
1.0	10	10	10	10	10	10	10	10	10	10	100.0
2.0	10	10	10	10	10	10	10	10	10	10	100.0
5.0	10	10	10	10	10	10	10	10	10	10	100.0
10.0	10	10	10	10	10	10	10	10	10	10	100.0
					Phorate sulfoxide						
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	0	0	0	0	0	0	0	0	0	0	0.0
0.1	0	0	1	0	0	0	0	0	0	0	1.0
0.2	2	0	3	2	3	3	2	4	1	0	20.0
0.3	3	2	3	2	4	3	2	6	2	2	29.0
0.4	7	3	6	6	5	9	6	10	9	9	70.0
1.0	10	7	9	8	8	10	9	7	9	8	85.0
2.0	10	10	10	10	10	10	10	10	10	10	100.0
5.0	10	10	10	10	10	10	10	10	10	10	100.0
10.0	10	10	10	10	10	10	10	10	10	10	100.0
					Phorate sulfone						
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	0	0	0	0	0	0	0	0	0	0	0.0
0.1	0	0	0	0	0	0	0	0	0	0	0.0
0.2	0	1	0	0	0	1	0	0	0	0	2.0
0.3	3	1	4	0	3	1	2	0	2	0	16.0
0.4	6	3	2	3	5	2	3	6	3	2	35.0
1.0	5	6	3	8	6	7	6	10	5	7	63.0
2.0	9	10	10	8	10	10	9	10	10	10	96.0
5.0	10	10	10	10	10	10	10	10	10	10	100.0
10.0	10	10	10	10	10	10	10	10	10	10	100.0

Table 12. Number of adult E. fabae dead within 48 hours of feeding on phoratoxon, phoratoxon sulfoxide, or phoratoxon sulfone in 1.5% sucrose (10 adults/cell)

Concentration (ppm)	Replicate feeding cells										Mortality %
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Phoratoxon											
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	0	0	0	0	0	0	0	0	0	0	0.0
0.1	0	0	0	0	0	0	0	0	0	0	0.0
0.2	2	0	0	1	0	1	2	0	1	0	7.0
0.3	2	0	1	0	1	2	0	1	3	2	12.0
0.4	5	5	4	5	5	5	4	4	4	4	45.0
1.0	10	10	8	10	9	9	9	8	8	9	90.0
2.0	10	10	10	10	10	9	9	10	10	9	97.0
5.0	10	10	10	10	10	10	10	10	10	10	100.0
10.0	10	10	10	10	10	10	10	10	10	10	100.0
Phoratoxon sulfoxide											
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	4	3	2	4	4	3	3	1	2	3	29.0
0.1	6	3	6	5	7	6	4	7	3	5	52.0
0.2	10	10	8	9	8	9	10	8	7	9	88.0
0.3	8	10	10	10	9	10	9	10	9	9	94.0
0.4	10	10	9	10	10	10	10	9	10	10	98.0
1.0	10	10	10	10	10	10	10	10	10	10	100.0
2.0	10	10	10	10	10	10	10	10	10	10	100.0
5.0	10	10	10	10	10	10	10	10	10	10	100.0
10.0	10	10	10	10	10	10	10	10	10	10	100.0
Phoratoxon sulfone											
0.016	0	0	0	0	0	0	0	0	0	0	0.0
0.08	1	0	2	0	1	0	1	1	0	2	8.0
0.1	2	2	1	2	1	1	4	2	2	1	18.0
0.2	10	5	5	6	5	7	10	9	7	10	74.0
0.3	6	9	9	10	8	8	10	10	10	6	86.0
0.4	10	8	10	9	9	10	9	9	9	10	93.0
1.0	0	0	0	0	0	0	0	0	0	0	100.0
2.0	0	0	0	0	0	0	0	0	0	0	100.0
5.0	0	0	0	0	0	0	0	0	0	0	100.0
10.0	0	0	0	0	0	0	0	0	0	0	100.0

Table 13. Number of adult E. fabae dead within 8 days on 0.08 ppm of each of phorate and phorate metabolites in 1.5% sucrose solution (5 adults/cell)

Day observed	Replicate feeding cells										Mortality %
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Phorate											
1	0	0	0	0	0	0	0	0	0	0	0.0
2	1	0	1	0	0	1	0	0	0	0	6.0
3	2	1	2	0	1	1	0	1	0	1	18.0
4	4	2	2	1	2	1	0	1	0	1	28.0
5	4	2	2	1	2	1	1	1	2	2	36.0
6	4	3	2	2	3	3	3	2	2	3	54.0
7	5	5	4	5	5	5	5	4	3	3	88.0
8	5	5	4	5	5	5	5	5	4	4	94.0
Phorate sulfoxide											
1	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	0	0	0	0	0	0	0	0	0.0
3	2	1	0	0	0	0	0	0	0	1	8.0
4	2	1	1	0	0	0	0	0	1	2	14.0
5	4	2	2	1	1	5	1	0	2	2	40.0
6	5	3	2	2	2	5	4	1	2	3	58.0
7	5	3	4	3	4	5	4	2	2	3	70.0
8	5	5	4	3	5	5	5	4	3	4	86.0
Phorate sulfone											
1	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	0	0	0	0	0	0	0	0	0.0
3	0	0	0	0	0	0	0	0	0	0	0.0
4	0	0	0	0	0	0	0	0	0	0	0.0
5	0	0	0	0	0	0	0	0	0	1	2.0
6	0	0	0	0	0	0	0	0	0	1	2.0
7	0	0	0	0	0	0	0	0	0	1	2.0
8	0	0	1	0	0	0	0	0	0	1	4.0

Table 13. (Continued)

Day observed	Replicate feeding cells I	II	III	IV	V	VI	VII	VIII	IX	X	Mortality %
					Phoratoxon						
1	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	0	0	0	0	0	0	0	0	0.0
3	0	0	0	0	0	0	0	0	0	0	0.0
4	0	0	0	0	0	0	0	0	0	0	0.0
5	0	0	0	0	0	0	0	0	0	0	0.0
6	0	0	0	0	0	0	1	0	0	0	2.0
7	0	0	0	0	0	0	1	0	0	0	2.0
8	0	0	0	0	0	0	1	0	0	0	2.0
					Phoratoxon sulfone						
1	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	1	0	0	1	1	0	0	1	8.0
3	0	3	3	1	2	3	4	2	2	2	44.0
4	2	3	3	2	5	4	5	4	3	4	70.0
5	3	3	5	4	5	4	5	4	5	4	84.0
6	5	4	5	5	5	5	5	5	5	5	98.0
7	5	5	5	5	5	5	5	5	5	5	100.0
					Phoratoxon sulfoxide						
1	0	0	0	0	0	0	0	0	0	0	0.0
2	3	2	2	3	3	4	2	3	2	2	52.0
3	5	5	5	4	4	5	4	5	5	4	92.0
4	5	5	5	5	5	5	4	5	5	5	98.0
5	5	5	5	5	5	5	5	5	5	5	100.0
6											
7											
					Sucrose (control)						
1	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	0	0	0	0	0	0	0	0	0.0
3	0	0	0	0	0	0	0	0	0	0	0.0
4	0	0	0	0	0	0	0	0	0	0	0.0
5	0	0	0	0	0	0	0	0	0	0	0.0
6	0	0	0	0	0	0	0	0	0	0	0.0
7	0	0	0	0	0	0	0	0	0	0	0.0
8	0	0	0	0	0	0	0	0	0	0	0.0

Table 14. Number of adult female T. urticae (5/cage) dead within 7 days on phorate-treated lima bean seedlings (roots submerged in phorate for 6 hours)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
		200 ppm						
1	I	0	3	3	3	3	3	3
	II	0	1	3	3	3	3	4
	III	0	2	4	4	4	4	4
	IV	0	2	2	2	3	3	3
2	I	1	1	3	4	5	5	5
	II	0	3	4	4	4	5	5
	III	1	3	3	4	4	4	4
	IV	1	2	5	5	5	5	5
3	I	0	1	1	2	3	4	4
	II	0	1	4	4	4	4	4
	III	1	1	4	4	4	5	5
	IV	0	2	5	5	5	5	5
4	I	0	1	2	2	4	4	5
	II	1	2	5	5	5	5	5
	III	2	4	5	5	5	5	5
	IV	1	2	4	4	4	5	5
5	I	0	4	4	4	4	4	4
	II	0	1	1	1	1	1	2
	III	1	1	2	3	3	4	5
	IV	3	4	4	5	5	5	5
		300 ppm						
1	I	0	0	2	5	5	5	5
	II	2	2	5	5	5	5	5
	III	0	1	3	3	5	5	5
	IV	2	4	5	5	5	5	5
2	I	0	1	2	2	3	3	3
	II	2	0	1	3	4	4	4
	III	0	1	2	3	3	3	3
	IV	0	2	4	4	5	5	5

Table 14. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
3	I	2	3	3	4	4	5	5
	II	0	2	4	4	4	5	5
	III	1	1	2	4	4	4	5
	IV	1	3	3	4	4	5	5
4	I	0	3	4	4	4	4	4
	II	3	3	5	5	5	5	5
	III	0	3	3	3	4	4	4
	IV	1	1	2	2	3	3	5
5	I	0	1	4	5	5	5	5
	II	2	2	3	4	4	5	5
	III	0	4	5	5	5	5	5
	IV	0	3	3	4	4	4	4
		400 ppm						
1	I	0	2	3	5	5	5	5
	II	1	3	5	5	5	5	5
	III	0	2	2	4	4	5	5
	IV	2	3	4	4	4	4	4
2	I	1	2	3	3	3	3	3
	II	1	2	2	4	4	5	5
	III	0	0	1	4	4	4	4
	IV	1	2	2	4	4	4	5
3	I	1	1	4	5	5	5	5
	II	0	3	4	5	5	5	5
	III	0	3	5	5	5	5	5
	IV	2	4	5	5	5	5	5
4	I	0	2	4	4	4	5	5
	II	0	1	2	3	3	3	3
	III	1	3	5	5	5	5	5
	IV	0	1	2	2	4	4	4
5	I	1	3	4	5	5	5	5
	II	0	3	3	4	5	5	5
	III	0	0	3	4	4	5	5
	IV	0	2	2	3	4	5	5

Table 14. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
		500 ppm						
1	I	1	1	4	5	5	5	5
	II	1	1	2	4	5	5	5
	III	0	3	4	5	5	5	5
	IV	0	3	4	5	5	5	5
2	I	2	5	5	5	5	5	5
	II	2	2	4	4	4	5	5
	III	1	2	4	4	4	5	5
	IV	3	5	5	5	5	5	5
3	I	1	3	4	4	5	5	5
	II	1	3	4	4	5	5	5
	III	2	3	3	4	5	5	5
	IV	1	3	4	4	5	5	5
4	I	0	0	2	5	5	5	5
	II	0	0	2	5	5	5	5
	III	3	3	4	4	4	5	5
	IV	2	2	2	4	4	5	5
5	I	0	0	3	3	5	5	5
	II	1	4	5	5	5	5	5
	III	2	4	4	5	5	5	5
	IV	0	1	4	4	4	5	5
		600 ppm						
1	I	0	2	2	3	4	4	4
	II	0	5	5	5	5	5	5
	III	1	2	4	5	5	5	5
	IV	0	2	3	4	5	5	5
2	I	1	4	5	5	5	5	5
	II	0	4	4	4	5	5	5
	III	1	2	3	4	5	5	5
	IV	0	4	5	5	5	5	5
3	I	1	2	3	4	5	5	5
	II	0	3	3	5	5	5	5
	III	1	2	3	4	5	5	5
	IV	0	2	5	5	5	5	5

Table 14. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
4	I	1	1	2	3	4	5	5
	II	0	3	4	5	5	5	5
	III	0	1	3	4	4	5	5
	IV	1	1	2	3	4	4	4
5	I	1	2	4	5	5	5	5
	II	1	3	4	5	5	5	5
	III	2	4	5	5	5	5	5
	IV	0	2	3	4	5	5	5
		Control						
1	I	0	0	0	0	0	0	1
	II	0	0	0	0	0	0	0
	III	0	1	2	2	2	2	2
	IV	0	0	0	0	0	0	0
2	I	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0
	IV	0	0	1	1	1	1	1
3	I	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	1
	III	0	0	1	2	2	2	2
	IV	0	0	0	0	1	1	1
4	I	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0
	III	0	1	1	1	2	2	2
	IV	0	0	0	0	0	0	0
5	I	0	0	0	1	1	1	1
	II	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0

Table 15. Number of adult female T. urticae (5/cage) dead within 7 days on phorate-treated lima bean seedlings (roots submerged in phorate for 3 hours)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
		200 ppm						
1	I	0	1	2	2	2	2	2
	II	2	2	2	2	2	2	2
	III	1	1	2	3	3	3	3
	IV	3	3	4	5	5	5	5
2	I	1	2	2	3	3	3	3
	II	0	0	0	0	1	1	2
	III	0	0	2	3	3	3	3
	IV	0	0	2	4	4	4	4
3	I	0	0	0	0	1	3	3
	II	1	1	1	1	1	1	2
	III	1	3	3	3	3	4	4
	IV	0	0	2	2	2	4	5
4	I	0	2	2	2	2	2	2
	II	0	0	0	1	1	2	2
	III	0	1	1	2	4	4	4
	IV	2	2	2	2	2	2	2
5	I	0	1	2	4	4	4	4
	II	0	1	2	2	2	3	3
	III	0	0	1	1	1	2	3
	IV	0	1	1	2	3	3	3
		300 ppm						
1	I	0	0	1	2	2	3	4
	II	0	1	2	3	4	5	5
	III	1	1	1	1	1	1	1
	IV	2	2	3	3	5	5	5
2	I	2	2	2	3	4	4	4
	II	1	2	4	4	5	5	5
	III	1	1	2	3	3	3	3
	IV	0	1	1	2	3	3	4

Table 15. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
3	I	1	2	3	5	5	5	5
	II	1	1	2	3	3	4	4
	III	0	0	0	0	0	0	0
	IV	0	0	1	1	1	1	3
4	I	0	1	2	2	3	3	3
	II	0	1	1	3	4	4	4
	III	0	1	2	3	4	5	5
	IV	1	1	1	3	3	5	5
5	I	0	0	1	3	3	4	5
	II	0	0	0	0	0	1	1
	III	1	1	1	1	2	2	3
	IV	1	2	2	2	3	3	3
		400 ppm						
1	I	0	0	0	0	1	1	2
	II	1	1	2	2	3	3	5
	III	1	3	3	4	5	5	5
	IV	0	0	1	2	2	3	3
2	I	0	0	1	3	3	4	4
	II	1	1	2	2	2	3	5
	III	0	2	4	4	4	5	5
	IV	1	2	3	3	4	4	5
3	I	0	2	4	4	5	5	5
	II	0	0	1	3	4	5	5
	III	0	1	1	2	2	3	3
	IV	1	2	3	4	4	5	5
4	I	1	2	2	2	2	2	5
	II	0	0	2	2	3	4	4
	III	0	2	2	2	3	3	3
	IV	1	1	1	1	2	2	2
5	I	0	0	1	4	4	4	5
	II	1	2	2	3	4	4	4
	III	0	1	1	1	2	3	3
	IV	0	1	2	2	2	2	3

Table 15. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
		500 ppm						
1	I	0	2	3	5	5	5	5
	II	1	1	5	5	5	5	5
	III	0	0	4	4	5	5	5
	IV	0	1	3	3	5	5	5
2	I	0	2	2	2	4	4	4
	II	0	1	2	3	3	3	3
	III	1	1	2	2	4	4	4
	IV	0	0	0	1	2	5	5
3	I	1	1	2	4	5	5	5
	II	1	3	3	3	5	5	5
	III	0	1	1	2	4	5	5
	IV	1	1	2	3	3	5	5
4	I	2	3	3	3	3	5	5
	II	1	1	1	4	5	5	5
	III	0	2	4	4	4	5	5
	IV	0	k	2	3	3	5	5
5	I	0	0	0	1	2	3	5
	II	0	1	1	1	2	4	4
	III	0	0	3	5	5	5	5
	IV	0	0	1	1	4	4	5
		600 ppm						
1	I	1	1	2	2	2	2	4
	II	0	0	0	1	2	5	5
	III	2	3	3	4	4	4	5
	IV	0	0	2	5	5	5	5
2	I	0	0	1	1	3	3	4
	II	0	0	0	0	5	5	5
	III	1	2	3	5	5	5	5
	IV	0	1	2	3	3	4	5

Table 15. (Continued)

Replicate plants	Replicate cages	Day observed						
		1	2	3	4	5	6	7
3	I	0	1	1	1	1	3	3
	II	0	0	2	3	4	5	5
	III	1	2	3	3	3	3	4
	IV	0	0	1	1	1	1	2
4	I	0	1	4	4	4	4	4
	II	0	1	3	3	3	3	4
	III	1	1	3	5	5	5	5
	IV	1	2	3	3	4	5	5
5	I	1	1	2	2	2	2	2
	II	0	0	1	2	3	3	3
	III	0	1	2	2	2	3	4
	IV	1	1	1	1	1	5	5

Table 16. Mortality of T. urticae on phorate treated lima bean seedlings; tests of significance based on variance† and paired comparisons of means

Dosage (coded)††	Day observed 1	3	5	7	Dosage (coded)††	Day observed 1	3	5	7
1 vs. 2	*	*	*	*	5 vs. 6	*	n.s.	n.s.	n.s.
1 vs. 3	*	*	*	*	5 vs. 7	*	*	*	*
1 vs. 4	*	*	*	*	5 vs. 8	*	*	*	*
1 vs. 5	*	*	*	*	5 vs. 9	*	*	*	*
1 vs. 6	*	*	*	*	5 vs. 10	*	*	*	*
1 vs. 7	*	*	*	*	5 vs. 11	*	*	*	*
1 vs. 8	*	*	*	*					
1 vs. 9	*	*	*	*	6 vs. 7	n.s.	*	*	*
1 vs. 10	*	*	*	*	6 vs. 8	n.s.	*	*	*
1 vs. 11	*	*	*	*	6 vs. 9	n.s.	*	*	*
					6 vs. 10	n.s.	*	*	n.s.
2 vs. 3	n.s.	n.s.	n.s.	n.s.	6 vs. 11	n.s.	*	*	*
2 vs. 4	n.s.	n.s.	n.s.	n.s.					
2 vs. 5	*	n.s.	*	*	7 vs. 8	n.s.	n.s.	n.s.	n.s.
2 vs. 6	n.s.	n.s.	*	*	7 vs. 9	n.s.	n.s.	n.s.	*
2 vs. 7	n.s.	*	*	*	7 vs. 10	n.s.	n.s.	*	*
2 vs. 8	n.s.	*	*	*	7 vs. 11	n.s.	n.s.	n.s.	*
2 vs. 9	n.s.	*	*	n.s.					
2 vs. 10	n.s.	*	n.s.	*	8 vs. 9	n.s.	n.s.	n.s.	n.s.
2 vs. 11	n.s.	*	*	n.s.	8 vs. 10	n.s.	n.s.	*	*
					8 vs. 11	n.s.	n.s.	n.s.	*
3 vs. 4	n.s.	n.s.	n.s.	n.s.					
3 vs. 5	n.s.	n.s.	*	*	9 vs. 10	n.s.	n.s.	*	*
3 vs. 6	n.s.	n.s.	*	*	9 vs. 11	n.s.	n.s.	n.s.	n.s.
3 vs. 7	n.s.	*	*	*					
3 vs. 8	n.s.	*	*	*	10 vs. 11	n.s.	n.s.	*	*
3 vs. 9	n.s.	*	*	*					
3 vs. 10	n.s.	*	*	n.s.					
3 vs. 11	n.s.	*	*	n.s.					
4 vs. 5	*	n.s.	*	*					
4 vs. 6	n.s.	n.s.	*	n.s.					
4 vs. 7	n.s.	*	*	*					
4 vs. 8	n.s.	*	*	*					
4 vs. 9	n.s.	*	*	*					
4 vs. 10	n.s.	*	n.s.	n.s.					
4 vs. 11	n.s.	*	*	*					

Table 16. (Continued)

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†Variance = npq where n, number tested; p, proportion dead; q, proportion alive.

††1, control:
2, 3, 4, 5, 6 roots submerged for 6 hrs in 200, 300, 400, 500, and 600 phorate concentrations:
7, 8, 9, 10, 11 roots submerged for 3 hrs in 200, 300, 400, 500, and 600 phorate concentrations.

* Significant at 95% probability.

n.s. Not significant.

Table 17. Cumulative % mortality of T. urticae exposed to treated lima bean seedlings (roots submerged 3 hr in 500 ppm phorate)

Mite groups	Day observed																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
G_0 †	1	19	38	48	68	84	91	95												
G_1 ††		7	12	16	20	25	29	35	39	42	52	58	58	59						
G_2			6	16	21	25	28	35	37	42	47	49	51	51	52					
G_3				3	8	14	19	28	32	37	38	45	47	51	55	55				
G_4					9	17	22	31	34	35	36	42	48	50	52	56	57			
G_5						15	19	25	27	29	31	33	37	40	43	44	45	45		
G_6							12	23	29	31	33	38	42	44	46	46	47	50	52	
G_7								12	15	18	24	25	30	32	34	35	37	40	41	42
G_8									15	20	20	30	36	39	43	43	45	48	48	49

†G_0 mites continuously on treated plants.

††G_1-G_8 mites exposed for 24 hr in successive days and transferred to untreated detached leaves.